Pharmacokinetics of sulfluramid and its metabolite desethylsulfluramid after intravenous and intraruminal administration of sulfluramid to sheep

Banjong Vitayavirasuk[1] and John M Bowen[2]*

[1]*Department of Pathology, Faculty of Medicine, Prince of Songkhla University, Hat Yai, Songkhla 90110, Thailand*
[2]*Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602–7371, USA*

Abstract: Pharmacokinetic properties and tissue residues of the insecticide sulfluramid (I) and its major metabolite desethylsulfluramid (II) were determined in healthy sheep after bolus intravenous (IV) administration (5 and 15 mg kg^{-1}; $n = 10$) and bolus intraruminal (IR) administration (100 and 400 mg kg^{-1}; $n = 12$) of I. Depression, lethargy, and dyspnea were noted for 4 h after the higher IV dose, but not after the other IV or IR doses. The time courses of the mean blood concentrations of I and II were best described by a two-compartment open model with rapid distribution and slow elimination phases. The blood-to-plasma concentration ratios for I and II were 1.43 (±0.50) and 26.7 (±9.41), respectively, suggesting binding of II to red blood cells. The $T_{1/2\beta}$ values for I and II for the higher IV dose of I were 15.3 (±4.68) h and 63.4 (±4.75) h and for the higher IR dose of I, 31.5 (±5.41) h and 74.9 (±7.49) h, respectively. Bioavailability was 28.6 (±2.96)% for the lower IR dose and 19.5 (±0.99)% for the higher IR dose. C_{max} values for II were higher in female than male sheep after IR administration of I. Only II was found in tissue samples, with the highest concentration being in liver (9.4 (±5.2) μg g^{-1}).
© 1999 Society of Chemical Industry

Keywords: sulfluramid; desethylsulfluramid; pharmacokinetics; sheep

1 INTRODUCTION

Sulfluramid (*N*-ethylperfluoro-octane-1-sulfonamide; $CF_3(CF_2)_7SO_2NHC_2H_5$; GX-071; certain Raid Max^R products) is a delayed-action insecticide that has toxicity against cockroaches,[1] termites, [2] argentine ants,[3] little fire ants,[4] and red imported fire ants.[5] Products for some of these applications are commercially available. Sulfluramid is structurally different from members of other classes of insecticides, and its long fluoride-saturated aliphatic chain makes it unique among the polyhaloalkanes. It is rapidly metabolized to desethylsulfluramid in rats and dogs.[6] Other metabolites have not been identified. Prolonged exposure of young rats to sulfluramid in their diet resulted in a transitory reduction in rate of weight gain.[7] In rabbit renal proximal tubule suspensions and isolated renal cortical mitochondria, sulfluramid and desethylsulfluramid uncoupled oxidative phosphorylation, which may provide a mechanism for mammalian toxicity.[8,9]

Interest in the large-scale application of sulfluramid to agricultural lands in a bait such as corn grits for control of the red imported fire ant[10] requires knowledge of its potential for toxicity to exposed grazing animals and occurrence of residues in food products derived from these animals. The purpose of this study was to characterize the pharmacokinetics of sulfluramid and its metabolite in sheep to assist in drawing conclusions regarding health risks associated with the application of sulfluramid to grazed pastures.

2 MATERIALS AND METHODS

2.1 Materials

Sulfluramid (**I**), desethylsulfluramid (**II**), and *N-tert*-butylperfluoro-octane-1-sulfonamide (internal standard) were provided by Griffin Corp, Valdosta, GA. The C_8 portion of **I** consisted of about 80% linear chain with the remainder being a mixture of branched-chain isomers. **II** and *N-tert*-butylperfluoro-octane-1-sulfonamide were also thought to contain a small percentage of branched-chain isomers. Polyethylene glycol 400 (PEG) was purchased from JT Baker Chemical Co, Phillipsburg, NJ. Ethyl acetate and

* Correspondence to: John M Bowen, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens GA 30602–7371, USA
E-mail: jbowen@peachnet.campus.mci.net
Contract/grant sponsor: University of Georgia Veterinary Medical Experiment Station, Environmental Toxicology Program
(*Received 17 July 1998; revised version received 14 December 1998; accepted 11 February 1999*)

© 1999 Society of Chemical Industry.

capillary GC grade hexane were obtained from Burdick and Jackson, Muskegon, MI. Collagenase, lipase, and protease enzymes and penicillin, streptomycin, and amphotericin B were obtained from Sigma Chemical Co., St Louis, MO.

2.2 Animals

Healthy mixed breed, young adult sheep (17 non-castrated males and 17 females) weighing between 27 and 35 kg were purchased from a local farm. The sheep were fed a fortified grain supplement, containing 15% protein. Hay and water were available *ad libitum*. During a one-week quarantine period, routine physical examination, blood examination (complete blood count and serum chemistry profile), vaccination, and deworming were performed on each sheep.

2.3 Intravenous administration

A dosing solution of 10 mg ml^{-1} **I** in PEG was prepared and warmed to 40 °C. The solution was administered as a rapid bolus at a dose of 5 mg kg^{-1} to five male and five female sheep at zero time through a catheter previously aseptically placed in the right external jugular vein. The catheter was removed after administration of **I**. Blood samples (0.2 ml each) were collected serially (see Figs 1 and 2 for intervals) over a 336-h period by direct venipuncture from the left external jugular vein. Each blood sample was placed in a citrated test tube and was frozen (−18 °C) until analysis. After a washout period (55 days), the same sheep were given a higher bolus intravenous (IV) dose (15 mg kg^{-1}) of **I** through a catheter aseptically placed in the right external jugular vein. The catheter was removed after administration. Serial blood samples (see Figs 1 and 2 for intervals) were collected as for the lower dose. The sheep were then euthanized with sodium pentobarbital. Tissue samples were collected from brain, adipose tissue, heart, kidney, liver, lung, skeletal muscle, and spleen. Each tissue sample was kept in a tightly sealed plastic bag and was frozen (−18 °C) until analyzed.

2.4 Intraruminal administration

A dosing solution of 25 mg ml^{-1} **I** in PEG was prepared and warmed to 40 °C. The solution was administered intraruminally at zero time via a 75-cm, 13-gauge syringe needle inserted through the left paralumbar fossa at dose rates of 100 and 400 mg kg^{-1} to 12 sheep (six males and six females) per dose level. Blood samples (0.2 ml) were collected by direct venipuncture over a 336-h period (see Figs 3 and 4 for intervals) from the external jugular vein. Blood samples were placed in citrated tubes and were frozen (−18 °C) until analysis. Additional samples of blood were obtained from each sheep at 12 h after dosing (400 mg kg^{-1}) for determination of the blood-plasma concentration ratio of **I** and **II**.[11] These samples were collected in citrated tubes. Plasma was obtained from one-half of each sample by centrifugation (2060 ***g***, 15 min) and was refrigerated. The whole-blood portion of each of these samples was frozen. The matched plasma and blood samples were analyzed within 12 h after being obtained, as described in Section 2.5. The sheep were euthanized at the end of the sampling period. Tissue samples, as in the bolus IV administration study, were collected from six sheep (three males and three females) for each dose for analysis of **I** and **II** concentrations.

2.5 Blood analyses

Blood samples were allowed to thaw once at room temperature which resulted in hemolysis of the red blood cells. After thawing of the blood samples was complete, the blood and plasma samples (0.2 ml) were spiked with acetone (0.02 ml) containing internal standard (100 μg ml^{-1}), and ethyl acetate (0.4 ml) was added. The samples were vortexed for 1 min, and then centrifuged at 2060 *g* for 20 min. The supernatant was collected from each sample for analysis of **I** and **II** using a gas chromatograph model HP5890A with electron-capture detector (GC/ECD) (Hewlett Packard Co, Avondale, PA). A 15-m × 0.53-mm fused silica column bonded with 1 μm thick polyethylene glycol (DBWAX) was used. The flow rate of carrier gas (helium) was 20 ml min^{-1}. Injections were done in splitless mode. The injection volume was 1 μl. Column temperature was programmed from 60 °C to 120 °C at 30 °C min^{-1} and from 120 °C to 220 °C at 12 °C min^{-1} with temperature remaining at 220 °C for 5 min. The assay was validated by measuring the concentration of known amounts of **I** and **II** in spiked blood samples. This procedure was repeated for each series of sample analyses and the results, adjusted for internal standard variations,[12] were used to establish a calibration curve. In a preliminary study, urine samples were collected after sheep were given a bolus IV dose (15 mg kg^{-1}) of **I**. Neither **I** nor **II** was detected in the samples using the blood assay procedure. Therefore, collection and analyses of urine were not included in this study.

2.6 Tissue analyses

The method of extraction of tissue samples was similar to that previously described.[7] A tissue sample weighing 0.5 g was cut into small pieces that were transferred to a 16 × 100 mm culture test tube. An enzyme mixture containing 6 mg ml^{-1} collagenase and 4 mg ml^{-1} protease was prepared. An aliquot of 1.5 ml of the enzyme mixture was added to each tissue sample except adipose tissue, to which 1.5 ml of enzyme mixture containing lipase (100), collagenase (2), and protease (1 mg ml^{-1}) was added. An aliquot of 50 μl of an antibiotic-antimycotic mixture of penicillin (5000 IU ml^{-1}), streptomycin (5 mg ml^{-1}), and amphotericin-B (10 mg ml^{-1})] was also added to each sample. The samples were incubated at 37 °C for 24 h and then extracted with ethyl acetate. After centrifugation at 2060 *g* for 30 min at 4 °C, the supernatant was removed and analyzed by GC/ECD with a 30-m × 0.53-mm fused silica column (DB5) bonded with a film thickness of 1 μm. The column temperature

was programmed from 65 °C to 200 °C at 12° min^{-1} and from 200 °C to 300 °C at 30 °C min^{-1} with the temperature remaining at 300 °C for 5 min. The flow rate of carrier gas (helium) was 20 ml min^{-1}. Injections were done in the splitless mode.

2.7 Data analysis

Kinetics of **I** and **II** were determined by fitting poly-exponential equations to the blood concentration-time data using nonlinear, least-square regression analysis (RSTRIP^R, Micromath Inc, Salt Lake City, UT). The best-fitting model was documented by applying the Model Selection Criterion to the data analysis for results for individual sheep. The kinetics for **I** and **II** after bolus IV administration of **I** were best evaluated using the equation

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

and the kinetics for **I** and **II** after bolus IR administration of **I** were best evaluated using the equation

$$C_t = Be^{-\beta t} - Be^{-\alpha t}$$

where A is the extrapolated blood concentration for $t = 0$ for the distribution–metabolite formation phase, B is the extrapolated blood concentration for $t = 0$ for the elimination phase, α is the apparent first-order distribution-metabolite formation rate constant, and β is the first-order elimination rate constant.[13,14] Calculated kinetic parameters included mean residence time in the body (MRT = AUMC/AUC), where AUC is the area under the concentration-time curve extrapolated to infinity, and AUMC is the area under the curve of a plot of the product of time and blood concentration versus time extrapolated to infinity,[15] total body clearance ($CL_b = Dose_{IV}/AUC$); distribution–metabolite formation phase half-life ($T_{1/2\alpha} = 0.693/\alpha$); elimination phase half-life ($T_{1/2\beta} = 0.693/\beta$); apparent volume of distribution in the central compartment ($V_c = Dose_{IV}/(A+B)$); apparent volume of distribution at steady state ($V_{SS} = MRT \times CL_b$); estimated blood concentration at $t = 0$ (C_0); estimated peak blood concentration (C_{max}); estimated time to peak blood concentration (T_{max}); estimated time between administration of **I** and its detection in blood or its metabolite's detection (lag time); and percentage of **I** absorbed or bioavailability ($F = AUC_{IR}/AUC_{IV} \times Dose_{IV}/Dose_{IR} \times 100$). Because a crossover design was not used, the mean value for the bolus IV administration of 15 mg kg^{-1} **I** was used in the calculation of F. The rate constants k_{12}, k_{21}, and k_{el}) were estimated from mean values using formulae described by Welling.[14] Unless noted otherwise, all pharmacokinetic parameters were calculated as mean values from results for individual sheep.

2.8 Statistical analysis

Student's t-test (two-tailed) was used to determine significant differences ($P < 0.05$). In a small number of comparisons where the variances were not equal or the distribution was not normal, a nonparametric test, the Mann–Whitney Rank Sum Test, was used. Data are expressed as means ± SD.

3 RESULTS

3.1 Assay validation

The peaks of internal standard, **I** and **II** were well separated in the chromatograms. Retention times for internal standard, **I** and **II** were 3.14, 3.97, and 8.32 min, respectively. The recoveries of internal standard, **I** and **II** over a range of 0.5–25.0 ng μl^{-1} in the chromatography samples were 96.9 (±5.54)%, 97.8 (±8.96)%, and 90.0 (±9.98)%, respectively, and were similar for the tissue assays, with the exception that the recovery of **II** was better (99.0 (±6.2)%). The coefficients of variation (CV) were 1.04–9.79% for **I** and 2.70–8.67% for **II**. The method detection limits for the blood assays for **I** and **II** were 0.3 and 0.54 μg ml^{-1}, respectively, and 0.3 μg g^{-1} for **I** and **II** in the tissue assays. Linear correlation coefficients of the calibration curves for the assays for **I** and **II** were routinely 0.999. Additional details on validation of the method for determination of **I** and **II** have been reported.[6,7,12]

3.2 Response of sheep to sulfluramid administration

Some minor toxic signs were seen in the sheep at the 15 mg kg^{-1} bolus IV dose of **I**. These signs occurred 10–15 min after dosing, lasted for about 4 h, and included depression, lethargy, and dyspnea. Signs of toxicity were not associated with the higher doses of **I** given by the IR route.

3.3 Intravenous administration

At the 5 mg kg^{-1} dose level, **I** could not be detected at 4 h post-administration (Fig 1). Because of the detection limit of the assay, the terminal slope of the blood concentration–time curve was absent, which limited determination of kinetic parameters for the elimination phase for **I** at this dose. At the 15 mg kg^{-1} dose level, the time course of blood concentration of **I**

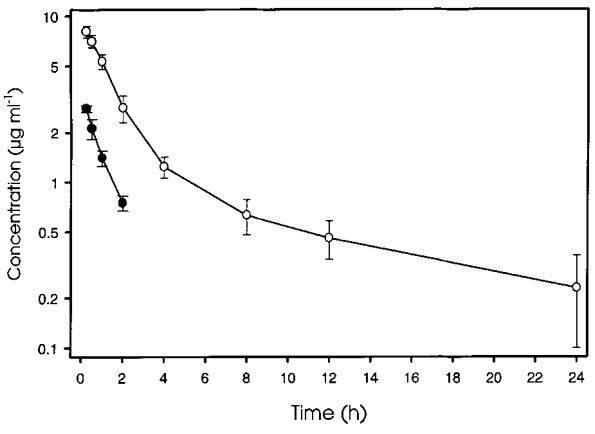

Figure 1. Blood concentration-time curve for sulfluramid after bolus IV administration of sulfluramid at (○) 15 and (●) 5 mg kg^{-1}. Bar = SD ($n = 10$).

Table 1. Pharmacokinetic parameters for sulfluramid and desethylsulfluramid concentrations after bolus intravenous administration of sulfluramid to sheep [n=10, means (±SD)]

	Dose and analyte			
Parameter	*5 mg kg^{-1} Sulfluramid*	*15 mg kg^{-1} Sulfluramid*	*5 mg kg^{-1} Desethylsulfluramid*	*15 mg kg^{-1} Desethylsulfluramid*
A (μg ml^{-1})	3.33 (±0.08)	8.78 (±0.59)	−20.9 (±1.74)	−37.5 (±2.94)[c]
α (h^{-1})	0.834 (±0.098)[a]	0.689 (±0.067)	0.966 (±0.496)	0.629 (±0.122)
B (μg ml^{-1})	—	0.810 (±0.204)	23.5 (±2.14)	61.4 (±5.57)[c]
β (h^{-1})	—	0.049 (±0.014)	0.0126 (±0.0027)	0.0111 (±0.0009)[c]
AUC (μg h ml^{-1})	4.04 (±0.46)[b]	30.4 (±6.83)	1933 (±499)	5572 (±839)[c]
MRT (h)	1.21 (±0.14)[b]	13.3 (±4.71)	74.5 (±6.75)	92.4 (±6.94)[c]
CL_b (litre h^{-1} kg^{-1})	1.25 (±0.15)[a]	0.514 (±0.100)	—	—
$T_{1/2\alpha}$ (h)	0.841 (±0.096)[b]	1.02 (±0.11)	0.87 (±0.36)	1.14 (±0.26)
$T_{1/2\beta}$ (h)	—	15.3 (±4.68)	50.9 (±4.78)	63.4 (±4.75)[c]
V_c (litre kg^{-1})	1.50 (±0.04)	1.57 (±0.11)	—	—
V_{ss} (litre kg^{-1})	1.50 (±0.04)	6.48 (±1.47)	—	—
C_o (μg ml^{-1})	3.34 (±0.08)	9.59 (±0.68)	—	—
C_{max} (μg ml^{-1})	—	—	21.8 (±2.11)	56.5 (±4.79)[c]
T_{max} (h)	—	—	5.07 (±1.65)	5.88 (±0.90)

[a] Female data significantly greater (P<0.05) than male data.
[b] Male data significantly greater (P<0.05) than female data.
[c] Significantly different (P<0.05) from results for lower dose (desethylsulfluramid results only).

was best fitted by a bi-exponential equation for all sheep (Fig 1; Table 1). **I** was rapidly converted to **II**, which was detected in the very first sample (0.25 h)(Fig 2). The time course of **II** blood concentration after the bolus IV administration of 5 and 15 mg kg^{-1} **I** for each sheep was best fitted by a bi-exponential equation (Fig 2; Table 1). Statistical comparison of the **II** pharmacokinetic parameters for the 5 and 15 mg kg^{-1} bolus IV doses of **I** indicated significant differences for A, B, β, AUC, MRT, $T_{1/2\beta}$, and C_{max} (Table 1). The first-order rate constants for distribution between central and peripheral compartments (k_{12} and k_{21}) were estimated to be 0.304 h^{-1} and 0.104 h^{-1}, respectively. The elimination rate constant (k_{el}) was estimated to be 0.332 h^{-1}.

3.4 Intraruminal administration

The time course of blood concentration of **I** after bolus IR administration of 100 or 400 mg kg^{-1} **I** for each sheep was best fitted by a bi-exponential equation (Fig 3; Table 2). Bioavailability was lower with the higher dose of **I**. The time course of **II** blood concentrations for each sheep was best fitted by a bi-exponential equation (Fig 4; Table 2). Statistical comparisons of the pharmacokinetic parameters for the 100 and 400 mg kg^{-1} bolus IR doses of **I** indicated significant differences for **I** in β, AUC, MRT, $T_{1/2\beta}$, lag time, C_{max}, and F and for **II** in B, β, AUC, MRT, $T_{1/2\beta}$, and C_{max} (Table 2). The blood-to-plasma concentration ratios for **I** and **II** were 1.43 (±0.50) and 26.7 (±9.41), respectively.

3.5 Sex differences

The results for the low bolus IV dose of **I** indicated a faster clearance of the compound in females, but the **II** pharmacokinetic parameters for this dose of **I** had no

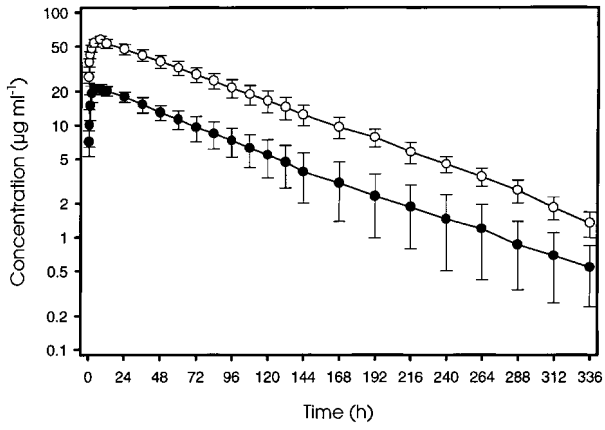

Figure 2. Blood concentration-time curve for desethylsulfluramid after bolus IV administration of sulfluramid at (○) 15 and (●) 5 mg kg^{-1}. Bar=SD (n=10).

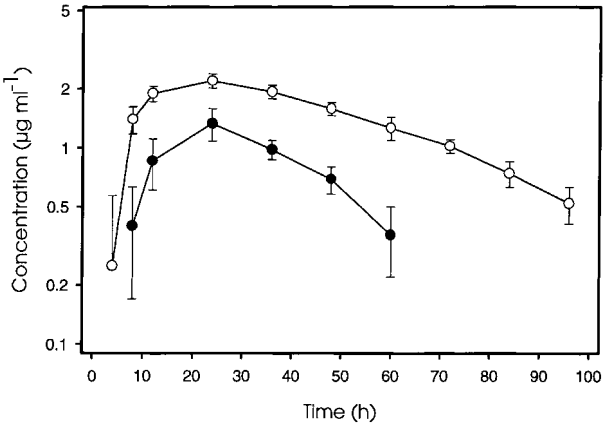

Figure 3. Blood concentration-time curve for sulfluramid after bolus IR administration of sulfluramid at (○) 400 and (●) 100 mg kg^{-1}. Bar=SD (n=12).

Table 2. Pharmacokinetic parameters for sulfluramid and desethylsulfluramid concentrations after bolus intraruminal administration of sulfluramid in sheep [n=12, mean (±SD)]

	Dose and analyte			
Parameter	*100 mg kg^{-1} Sulfluramid*	*400 mg kg^{-1} Sulfluramid*	*100 mg kg^{-1} Desethylsulfluramid*	*400 mg kg^{-1} Desethylsulfluramid*
B (μg ml^{-1})	6.90 (±2.40)	5.48 (±3.21)	66.1 (±12.6)[a]	126 (±22.7)[c]
α (h^{-1})	0.0848 (±0.0124)	0.1094 (±0.0477)	0.0463 (±0.0048)	0.0475 (±0.0107)
β (h^{-1})	0.0476 (±0.0073)	0.0227 (±0.0044)[c]	0.0110 (±0.0008)	0.0094 (±0.0009)[b,c]
AUC (μg h ml^{-1})	57.9 (±6.02)	158 (±7.93)[c]	4597 (±858)[a]	10904 (±2520)[c]
MRT (h)	33.4 (±3.82)	56.0 (±4.42)[c]	114 (±6.22)	130 (±12.0)[a,c]
$T_{1/2\alpha}$ (h)	8.32 (±1.06)	7.56 (±3.40)	15.1 (±1.61)	15.2 (±2.76)
$T_{1/2\beta}$ (h)	14.9 (±2.36)	31.5 (±5.41)[c]	63.6 (±4.55)	74.9 (±7.49)[a,c]
Lag time (h)	6.12 (±2.00)[b]	2.37 (±1.90)[c]	2.52 (±1.76)[b]	2.67 (±0.68)
C_{max} (μg ml^{-1})	1.31 (±0.22)	2.25 (±0.18)[c]	31.9 (±4.86)[a]	72.0 (±11.3)[a,c]
T_{max} (h)	21.8 (±2.67)[b]	21.5 (±2.05)	43.5 (±2.52)	46.2 (±5.76)
F (%)	28.6 (±2.96)	19.5 (±0.99)[c]	—	—

[a] Female data significantly greater (P<0.05) than male data.
[b] Male data significantly greater (P<0.05) than female data.
[c] Significantly different (P<0.05) from results for lower dose.

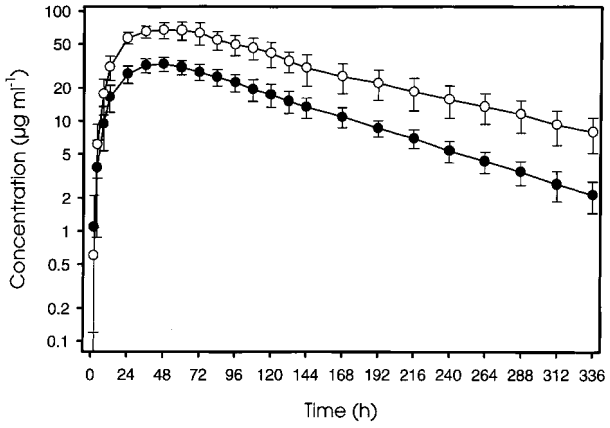

Figure 4. Blood concentration-time curve for desethylsulfluramid after bolus IR administration of sulfluramid at (○) 400 and (●) 100 mg kg^{-1}. Bar=SD (n=12).

sex differences. The results for the higher bolus IV dose of **I** revealed no sex differences for the pharmacokinetic parameters for **I** or **II**. The results for the lower and higher bolus IR doses of **I** showed little or no sex dependency of levels of **I**, but the levels of **II** associated with these doses of **I** had a significantly higher C_{max} in females.

3.6 Tissue residues

Tissue concentrations of **II** 14 days after administration of **I** are presented in Table 3. **I** was not detected in any of the tissues sampled. Highest tissue concentrations after bolus IV administration of **I** (15 mg kg^{-1}) were found in liver, spleen, and kidney, in order of decreasing concentration. After bolus IR administration, the highest concentrations were noted in liver, kidney, and lung, in order of decreasing concentration. Only for the liver and kidney samples were all samples positive for **II** (Table 3). For the higher bolus IR dose, two-thirds of the brain and muscle samples had detectable concentrations of **II**, but these concentrations were 28% and 21%, respectively, of liver concentrations in the same sheep.

4 DISCUSSION

II appeared to be the major metabolite of **I** in sheep, as

Table 3. Tissue desethylsulfluramid concentrations 14 days after bolus intravenous or intraruminal administration of sulfluramid

		Tissue concentration (μg g^{-1})							
Dosage and route	*N*	*Brain*	*Adipose tissue*	*Heart*	*Kidney*	*Liver*	*Lung*	*Muscle*	*Spleen*
15 mg kg^{-1} IV	10	0.3 (±0.0)[a] (3)[b]	0.3 (±0.1) (3)	0.3 (1)	1.3 (±0.7) (10)	4.8 (±4.0) (10)	0.7 (±0.4) (5)	0.3 (1)	1.5 (±1.0) (8)
100 mg kg^{-1} IR	6	1.4 (2)	0.4 (2)	— (0)	3.2 (±1.5) (6)	6.5 (±4.5) (6)	2.0 (±0.2) (4)	0.5 (1)	— (0)
400 mg kg^{-1} IR	6	2.6 (±2.4) (4)	0.6 (±0.2) (3)	— (0)	7.7 (±4.4) (6)	9.4 (±5.2) (6)	4.5 (±3.8) (6)	2.0 (±1.2) (4)	— (0)

[a] Mean (±(SD); the standard deviation was calculated when three or more detectable concentrations were obtained.
[b] Number of samples with detectable concentrations; these were used to calculate mean values.

has been reported for rats and dogs.[6,7,16] Because neither compound was identified in urine, the kidney does not appear to play a significant role in the elimination of **I** or its metabolite. The C_8 perfluorinated chain and the —SO_2—NH— group are generally considered to be resistant to biotransformation.[17] Evaluation of gas chromatograms and mass spectra of blood extracts from rats and dogs given oral doses of **I** did not reveal metabolites of **I** other than **II**.[6] Biotransformation of **I** to **II** is believed to involve a cytochrome P450-mediated *N*-dealkylation that occurs primarily in the liver.[16] Liver was found to be the tissue with the highest residue of **II** in the present study. Ruminants are considered to have highly efficient biotransformation mechanisms, including those associated with P450 systems.[18] The concentration of **II** very rapidly exceeded that of **I**, regardless of the route of administration or dose. The ratios of **II** to **I** for C_{max} values for the 100 and 400 mg kg^{-1} bolus IR doses of **I** were 31.9/1.31 and 72.0/2.25, respectively (Table 2). The magnitude of these ratios indicates existence of an efficient first-pass effect with a high liver extraction ratio. Body clearance (0.514 litre h^{-1} kg^{-1}; Table 1) of **I** after its bolus IV administration (15 mg kg^{-1}) was considerably less than the normal total hepatic blood flow of 2.4 litre h^{-1} kg^{-1} reported for sheep.[19] This difference suggests that the pathway for biotransformation of **I** to **II** would not be saturable at the doses used in the present study.

Biotransformation is also believed to enhance toxicity, because **II** is three times more potent than **I** as an uncoupler of oxidative phosphorylation.[9] In the present study, toxicity ended about 4 h after bolus IV administration of **I** (15 mg kg^{-1}). Because termination of signs of toxicity occurred prior to the T_{max} (5.88 h) for formation of **II** (Table 1), other factors may contribute to toxicity. Toxicity did not occur after IR administration even though the doses of **I**, when adjusted for bioavailability, were higher than the IV dose causing toxicity and the C_{max} values for **II** were higher after bolus IR administration than after bolus IV administration (Tables 1 and 2). However, the levels of **I** were lower after bolus IR administration than after bolus IV administration (Figs 1 and 3) which suggests that **I** does contribute directly to the toxicity. The finding of a high blood-to-plasma concentration ratio for **II** suggests that the latter is bound to red blood cells, which may provide a protective effect *in vivo*. Studies in rats revealed that **II** is also highly bound to plasma proteins (Vitayavirasuk B, unpublished), which could also have a protective effect.

Elimination of **II** by excretion in bile as a glucuronide conjugate has been demonstrated to occur in rats (Vitayavirasuk B, unpublished). Sulfonamide compounds with molecular masses greater than 325 (**I** = 527; **II** = 499) commonly undergo phase II biotransformation via *N*-glucuronidation in the liver with the product, a β-glucuronide conjugate, being excreted in bile.[20] Sheep are believed to fall into an intermediate class as biliary excretors of chemicals.[21] The long $T_{1/2\beta}$ for **II** in sheep might be the result of enterohepatic recycling which would involve release of the compound from the conjugate by action of β-glucuronidases in the intestinal tract and subsequent absorption of the freed **II**. Eventual elimination of **II** from the body would be through excretion of the conjugated and free forms in the feces.

Several differences in pharmacokinetic parameters associated with increase in dosage were significant (Tables 1 and 2). Most of these differences can be directly attributed to the increase in dose, eg AUC and C_{max}. However, the significant increases in $T_{1/2\beta}$ after bolus IV and IR administration of **I** suggest that a saturable pathway or pathways may be involved. For bolus IV admnistration, this pathway may be the conversion of **I** to **II**. The $T_{1/2\beta}$ for **I** after bolus IR administration may be influenced by dose differences because of the poor aqueous solubility of **I**, resulting in a non-uniform dissolution of the latter in the fluids of the digestive tract and alteration in its time course of absorption. This could affect calculation of the terminal slope of the concentration–time curve for **I** and, thus, the $T_{1/2\beta}$ value. Adjustment of the bolus IR dose of **I** for bioavailability indicated that the sheep were still exposed to higher doses of **I** than the higher bolus IV dose, although the time course of exposure was slower and the C_{max} was lower. These results for the bolus IR dose suggest that the conversion of **I** to **II** by first-pass metabolism has a large clearance capacity. In addition to the effect of possible enterohepatic recycling, the increase in $T_{1/2\beta}$ for **II** might occur through saturation of the pathway for conjugation of **II** and the active secretion of the conjugate into bile.

In view of the small number of significant sex differences (Tables 1 and 2), sex appeared to have very little effect on pharmacokinetics of **I** and **II**. Parameters with sex differences in sheep were not consistent for the different doses and metabolite levels, with the exception that the C_{max} values for females for **II** were about 24% higher at both the 100 and 400 mg kg^{-1} bolus IR doses of **I**. These results suggest a more rapid biotransformation of **I** to **II** in female sheep, but this could also be associated with slower elimination because the MRT and $T_{1/2\beta}$ were higher in females than males, at least at the 400 mg kg^{-1} bolus IR dose (Table 2). Studies in rats have suggested that females metabolize **I** at a faster rate than males.[16]

The concentration of **I** used for field assays for evaluation of the efficacy of the compound against the red imported fire ant is about 2 g acre^{-1}.[10] If a 30-kg sheep consumed the entire treated bait applied to one acre, the dose would be 67 mg kg^{-1}, which would not be expected to produce any toxicity, considering the low efficiency of ingestion and the long time required for ingestion of the bait by grazing this pasture area. Consumption of 1 kg of the treated corn grit bait in bulk form would result in an exposure dose of about 250 mg kg^{-1}. Although this concentration would not be expected to produce toxicity, residues of **II** would

probably be present in some of the edible tissues. Based on the $T_{1/2\beta}$ value for **II** in blood at the 400 mg kg^{-1} dose of **I**, it can be estimated that an additional 300 h (12 days) would be required for reduction of blood concentration to non-detectable levels, 375 h (16 days) for reduction of liver concentration to non-detectable levels, and 150 h (6 days) for reduction of muscle concentration to non-detectable levels if the muscles had residues of **II**.

In rats exposed to **I** in their diet (75 mg kg^{-1} feed) for 56 days, only **II** was detected in blood and tissues,[7] as was true for tissues in this sheep study. The $T_{1/2\beta}$ values for **II** for rat blood and liver were 10.8 days and 6.9 days, respectively. The rat blood $T_{1/2\beta}$ is much longer than that for sheep in the present study, suggesting that enterohepatic recycling of **II** may be more efficient in the monogastric animal. Rats have been classified as good biliary excretors,[21] which may be accompanied by enhanced recycling. A $T_{1/2\beta}$ value could not be determined for rat muscle because concentrations of **II** were below the detection limit. Concentrations in seven of 12 sheep muscle samples were also below the detection limit (Table 3). Actual $T_{1/2\beta}$ values for edible tissues need to be established to enable definition of withdrawal periods for sheep exposed to **I. II** appears to have a low affinity for adipose tissue in rats[7] and in sheep.

ACKNOWLEDGEMENTS

This research was supported by a grant from the University of Georgia Veterinary Medical Experiment Station's Environmental Toxicology Program.

REFERENCES

1 Schal C, Sulfluramid resistance and vapor toxicity in field-collected German cockroaches (Dictyoptera: Blattellidae). *J Med Entomol* **29**:207–215 (1992).
2 Su NY, Scheffrahn RH and Ban PM, Effects of sulfluramid-treated bait blocks on field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *J Econ Entomol* **88**:1343–1348 (1995).
3 Blachly JS and Forschler BT, Suppression of late-season Argentine ant (Hymenoptera: Formicidae) field populations using a perimeter treatment with containerized baits. *J Econ Entomol* **89**:1497–1500 (1996).
4 Williams DF and Whelan PM, Bait attraction of the introduced pest ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae) in the Galapagos Islands. *J Entomol Sci* **27**:29–34 (1992).
5 Vander Meer RK, Lofgren CS and Williams DF, Control of *Solenopsis invicta* with delayed-action fluorinated toxicants. *Pestic Sci* **17**:449–455 (1986).
6 Arrendale RF, Stewart JT, Manning R and Vitayavirasuk B, Determination of GX-071 and its major metabolite in rat blood by cold on-column injection capillary GC/ECD. *J Agric Food Chem* **37**:1130–1135 (1989).
7 Grossman MR, Mispagel ME and Bowen JM, Distribution and tissue elimination in rats during and after prolonged dietary exposure to a highly fluorinated sulfonamide pesticide. *J Agric Food Chem* **40**:2505–2509 (1992).
8 Schnellmann RG, The cellular effects of a unique pesticide sulfluramid (*N*-ethylperfluorooctane sulfonamide) on rabbit renal proximal tubules. *In Vitro* **4**:71–74 (1990).
9 Schnellmann RG and Manning RO, Perfluorooctane sulfonamide: a structurally novel uncoupler of oxidative phosphorylation. *Biochim Biophys Acta* **1016**:344–348 (1990).
10 Williams DF, Lofgren CS and Vander Meer RK, The red imported fire ant, *Solenopsis invicta*: Control with fluoroaliphatic sulfone bait toxicants. *J Agric Entomol*, **4**:41–47 (1987).
11 Klotz U, Estimation of the blood-plasma concentration ratio of diazepam in the rat. *J Pharmacokinet Biopharm* **13**:347–348 (1985).
12 Arrendale RF, Stewart JT, Mispagel ME and Vitayavirasuk B, Comparison of cold on-column and splitless injection using an automatic liquid sampler: Application to the determination of GX-071 in animal ration. *J High Resolut Chromatog* **12**:749–752 (1989).
13 Lanusse CE, Gascon LH and Prichard RK, Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. *J Vet Pharmacol Ther* **18**:196–203 (1995).
14 Welling PG, *Pharmacokinetics: Processes, Mathematics, and Applications*, 2nd edn, American Chemical Society, Washington, DC. pp 277 & 282 (1997).
15 Gibaldi M and Perrier D, *Pharmacokinetics*, 2nd edn, Marcel Dekker, Inc., NY. pp 175, 445–449 (1982).
16 Manning RO, Bruckner JV, Mispagel ME and Bowen JM, Metabolism and disposition of sulfluramid, a unique polyfluorinated insecticide, in the rat. *Drug Metab Dispos* **19**:205–211 (1991).
17 Johnson JD, Gibson SJ and Ober RE, Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C] perfluorooctonate or potassium [^{14}C]perfluorooctanesulfonate. *Fundam Appl Toxicol* 4:972–976 (1984).
18 Baggot JD, *Principles of Drug Disposition in Domestic Animals*, WB Saunders Co, Philadelphia. pp 160–166 (1977).
19 Smith CR and Hamlin RL, Regional circulation, in *Duke's Physiology of Domestic Animals*, 9th edn, ed by Swenson MJ, Cornell University Press, Ithaca, NY. p 134 (1977).
20 Millburn P, Smith RL and Williams RT, Biliary excretion of foreign compounds. *Biochem J* **105**:1275–1281 (1967).
21 Williams RT, Species variations in drug biotransformations, in *Fundamentals of Drug Metabolism and Disposition*, ed by LaDu BN, Mandel HG and Way EL, Williams & Wilkins, Baltimore. pp 187–205 (1971).